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Zoomicrobial Indicators of Water Pollution

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HISTORICAL BACKGROUND

The use of microscopic animals as indicators of water pollution has its history as far back as near the end of the 19th century when Rafter (1889) indicated, in his Discussion of a paper on self-purification of streams by Currier (1891), that certain "minute animals" played an important role in the self-purification process. It was, however, Kolkwitz and Marsson (1902, 1908, 1909, 1911) who introduced the "saprobity" system to define the zones of a sewage-polluted stream during self-purification and developed an ecological classification of microorganisms for the purpose of zoning such streams (Kolkwitz and Marsson (1908, 1909)).

Further elaboration and refining of the "saprobity" system were made later by Kolkwitz (1935, 1950) and by Liebmann (1951, 1962), and modified by workers in Central Europe (Dittmar, 1959; Gabriel, 1946; Knöpp, 1954; Kothé, 1962; Pantle & Buck, 1955a, 1955b; Sládeček, 1961; Šrámek-Hušek, 1956; Zelinka et al., 1959; Zelinka & Marvan, 1961), by Fjerdingstad (1960) in Norway, and by Horasawa (1956) in Japan. With the exception of

Herasawa's method, all the different versions of the "saprobit" system are based on numerical values assigned to different pollution indicator organisms listed by Liebmann (1951, 1962).

The *saprobien index* developed by Pantle and Buck has been adapted in East Germany as a standard measure of water pollution (Breitig, 1961); it has the formula

$$S = \frac{\sum s \cdot \sum h}{\sum h}$$

where S is the *saprobien index*, s is a numerical value obtained from Liebmann's list for pollution indicator organisms, and h is the frequency of occurrence of such organisms. This method, therefore, requires a lengthy and detailed examination of water by a competent protozoologist.

Šrámek-Hušek (1956), Sládeček (1961), and others introduced an expanded "saprobit" system which includes industrial wastes and more zones of pollution. The toxic effects of industrial wastes were taken into consideration, and so was the radioactivity.

Designating bacteria as reducers (R), algae as producers (P), and ciliates as consumers (C), Gabriel developed the R-P-C system. A BIP (biological index of pollution) is computed from the numerical values of each of these 3 groups of organisms.

The BIP system developed by Kothé was based on the decrease in the number of species of microbes in polluted waters. His formula for computing the BIP is as follows:

$$BIP = \frac{A_1 - A_x}{A_1} \times 100$$

where A_1 is the number of species of organisms in water above the point of discharge and A_x is the number of species below this point.

A value of zero for BIP means no reduction in the number of species, hence, absence of pollution and a value of 100 means complete absence of species below the point of discharge and, therefore, gross pollution.

Horasawa's method for determining the BIP is relatively simple in that no identification of species is required and the BIP is obtained by the formula

$$BIP = \frac{B}{A + B} \times 100$$

where A is the total number of chlorophyll-bearing one-cell organisms and B is the total number of nonpigmented protozoa. The water is classified according to the values obtained for BIP in this manner:

0 - 8	= clear water
9 - 20	= slightly polluted
21 - 60	= moderately polluted
61 - 100	= grossly polluted

The method developed by Fjerdingstad for measuring degree of pollution differs from those described above in that it is based on the composition of the benthic microbial communities. By this method, the author defined 9 levels of pollution and extended the evaluation to include effectiveness of correctional measures. Fjerdingstad's method gained support in some European countries because the benthic communities are more stable and the method involves smaller number of indicator organisms.

In the first edition of International Standards for Drinking Water, published by the World Health Organization (1958), the subject on biological indicators of pollution was included, and Horasawa's method for determining BIP was given in detail. In the second edition (1962), Horasawa's method was replaced by those developed by Gabriel (1946), Sládeček (1961), Fjerdingstad (1960), and Kothé (1962). The third edition published recently (1971) has omitted the specific methods and gives a general

account of the value of biological examination, especially of waters that are not subjected to standard treatment in supply systems.

The Standard Methods for the Examination of Water and Wastewater, published by the American Public Health Association, American Water Works Association, and Federation of Water Pollution Control, have excluded any method for determining biological indicators of water pollution. A chapter on Biological Sampling and Analysis was included in the 10th edition (1955), however. The analysis is limited to plankton population measured in volume-units mainly for the purpose of control of odor and taste due to the algal growth. Of particular interest is the fact that the 13th edition of the Standard Methods (1971) devotes one seventh of its space to biological sampling and analysis, and emphasizes on reporting plankton in categories of non-flagellated protozoa, flagellated protozoa, rotifers, etc., without mentioning nematodes, and no particular significance is attached to the presence of zoo-microbes in relation to pollution of water.

THE ROLE OF ZOOMICROBES AS INDICATORS OF WATER POLLUTION TODAY

It would seem a step backward in water hygiene to consider the use of zoomicrobes as pollution indicators when the water examination stresses on expediency and accuracy combined, with increasing interest in developing procedures for virological evaluation. There is no doubt that enteric bacteria and viruses are direct indicators of water pollution; they present a cross-

section picture of, but tell very little of, the events in the history of pollution.

Most of the municipal supply systems in this country today use surface waters that have been employed as carriers of effluents of domestic sewage and/or industrial wastes. It is important to know the health hazard potential of such waters in disseminating infectious agents, and it is also important to know the state of these waters in their function as carriers of waste effluent, negligence of which may result in a harmful effect on the ecological system. Such knowledge can be, at least, partially obtained by combining a zoomicrobial analysis with the bacteriological (and/or virological). For instance, a coliform count of 5,000 per 100 ml of water associated with very few zoomicrobes would indicate the occurrence of some unusual events in the receiving water, unless the effluent has been subjected to stabilization-pond treatment which encourages algal, but suppresses zoomicrobial, growth or comes from an anaerobic treatment process.

Zoomicrobes, furthermore, deserve attention in their own right in water examination. Free-living nematodes have been found in large numbers in a municipal supply (Chang et al., 1959). A survey of free-living nematodes and amoebae in municipal supplies revealed the presence of both zoomicrobes in small numbers in those supplies using polluted surface waters as raw sources (Chang et al., 1960). Most of the free-living nematodes in polluted surface waters have been traced to the sewage effluents they received (Chang & Kabler, 1962; Chaudhuri et al.,

1964, 1965). Free-living nematodes isolated from municipal supplies have been found capable of ingesting human enteric pathogens under laboratory conditions and protecting them against chlorination (Chang et al., 1960).

Examination of nematodes concentrated from sewage effluents revealed the presence of *E. coli* and streptococci, and the coliform MPN (most probable number) was highest in nematodes from the effluent of trickling filters and least from that of stabilization ponds (Chang and Kabler, 1962).

Under conditions close to field than laboratory, *Diplogasteroides* nematodes were found to carry enteroviruses at a concentration of about 1 PFU (plaque forming unit) per 250 worms (Chang, 1970). If a virus concentration of 1 per 50 gallons of finished water is considered unsafe (Coin, 1967; Berg, 1971), the presence of an average of 5 nematodes in a gallon of finished water (Chang et al., 1960) deserves attention from virological consideration, because there is a possibility that one of the estimated 250 nematodes in a 50-gal volume of finished water may harbor a virus. Its significance is increased by the fact that the nematode carried virus is protected from chlorination.

Recently, the free-living amoeba, *Naegleria*, has been found to cause fatal cases of meningoencephalitis, and all but one of the cases in the U.S. have been associated with swimming in small warm lakes (Chang, 1971). Although the pathogenic *Naegleria* has been shown to be different in cultural and animal pathogenicity aspects from the common *N. gruberi* found in sewage

effluent surface waters, its absence in sewage effluent remains to be ruled out (Chang, 1972).

From the afore-presented facts, it is clear that the zoomicrobes play a significant role in water hygiene, and their inclusion in water examination is justified. The question remaining is can a practical method be developed for zoomicrobial analysis. In the sections that follow, it will be seen that a practical method is available for such an analysis and the ZIP (zoomicrobial index of pollution) with emphasis on presence of predominant group(s) of zoomicrobes is useful in supplementing the information on pollutional effects on water quality.

A METHOD FOR EXAMINING ZOOMICROBES IN WATER

AS POLLUTION INDICATORS

The various methods or systems for determining the BIP afore-described are unsuitable for examination of water today because the surface waters are used, as stated before, as carriers of waste effluents. The "foreign" organisms found in the receiving waters are those already present in the effluent. Due to the drastic reduction in bacteria-food, most of the large ciliates, such as *Paramecium*, *Spirostomum*, *Colpidium*, and stalked ciliates which thrive in aerobic sewage treatment processes, disappear from the receiving water within a matter of hours. Small zoomicrobes generally survive longer periods of time in receiving waters and nematodes and protozoan cysts survive the longest, in days for the former and weeks for the latter. Rotifers, such as *Philodina* and *Monostyla* which feed mainly on bacteria (Chang, 1970) and flourish in aerobic sewage

treatment, have surviving times somewhere between these 2 groups. The large zoomicrobes are poor swimmers and susceptible to settling, the rate of which depends on the flow-rate of the receiving water.

Other effluent-bound zoomicrobes which deserve mentioning are the microscopic mites and bristle worms; their numbers are very small in comparison with the protozoa and nematodes.

In the study of nematodes in effluents from aerobic sewage treatment plants (Chang & Kabler, 1962), the effluents were also examined for zoomicrobes other than the nematodes. The major groups found in the examination in order of frequency are: small amoebae, ciliates, nematodes, rotifers, flagellates (mostly *Bodo*, *Paranema*, and *Monas*), and large amoebae (Author's unpublished data). Mites and bristle worms were found in larger numbers in trickling filter effluents, but the numbers were still small in comparison with those mentioned above.

When an effluent is discharged into a body of water, the zoomicrobes in the former will decrease continually as the water is moved farther away from the point of discharge. The rate of decrease in the zoomicrobial population in a stream reflects the flow time and flow rate, and that in a stagnant body of water reflects the detention time which controls settling and dispersion, excluding any toxic effect. The predominance of any particular group or groups in the receiving water reflects the nature of the effluent and/or the existence of ecological factors that influence the survival or presence of one group over the other.

Relatively clean surface waters generally have plankton dominated by chlorophyll-bearing organisms. Palmer (1967a,b,c)

reported the presence of algae in numbers over 1,000 per ml in surface waters in several states, and phytoflagellates, such as *Synura*, *Euglena*, *Eudorina*, and *Dinobryon* were present in numbers significantly greater than that of zoomicrobes normally encountered in relatively clean waters.

A survey of rotifers in a large number of rivers in the United States (Williams, 1966) revealed that the abundant members fell into 5 genera, all of which are planktonic in habitat and, therefore, uncommon in sewage effluent. These data suggest that in the use of zoomicrobes as pollution indicators, the inclusion of rotifers would introduce an error in the interpretation, unless the procedure involves identification of species which is too time-consuming in a routine examination of water.

During the survey of free-living nematodes and amoebae in municipal supplies (Chang et al., 1960), samples of raw water were also collected and examined for nematodes and other zoomicrobes. The results revealed a few interesting facts: (1) Nonpigmented protozoa outnumber the nematodes by a wide margin. (2) The rotifer population consists mostly of those genera reported by Williams (1966), and very few are commonly found in sewage effluents. (3) Mites and bristle worms are very rare in surface waters. (4) Practically no nematodes and small numbers of nonpigmented protozoa are present in clean or remotely polluted waters; the numbers of these 2 groups of zoomicrobes increase steadily with the increase in degree of pollution. (5) Of the protozoa group, small amoebae are the most abundant.

flagellates less, and ciliates the least (author's unpublished data).

Taking all these facts into consideration, a definite population size relationship exists between the phytoflagellates and the nonpigmented protozoa-nematode, on the basis of which a zoomicrobial pollution index (ZPI) has been formulated as follows:

$$ZPI = \frac{A + B + 1,000C}{A}$$

where A is the total number of phytoflagellates, B is the total number of nonpigmented protozoa, and C is the number of nematodes. The figure 1,000 is used as a weighing factor for the importance of the occurrence of nematodes as a pollution indicator. This value was arrived at after a study of more than 50 rivers and 5 lakes; most of the rivers and two of the lakes were polluted by effluents from aerobic treatment plants.

To show the background knowledge to the development of the formula, the population distributions of the 3 categories of organisms in these surface waters are presented in Table 1. The ratios between the protozoa and nematode counts were also computed and shown in the same Table.

Table 1 shows that the protozoa-nematode population in clean and in remotely polluted waters amounts to only a small fraction of that of the phytoflagellates. As the level of population increases, there is a corresponding decrease in phytoflagellates and a similar increase in protozoa-nematodes

in the water. The smaller population of nematodes in polluted lakes than in polluted rivers can be explained by the faster settling of nematodes in still or stagnant, than in flowing waters. Of particular interest is the fact that the protozoa:nematode population ratio in rivers decreases significantly with each increase in the degree of pollution.

From the enumerational relationship between the counts of the phytoflagellates and the protozoa-nematodes presented in Table 1, a scale of values is proposed for measuring the levels of pollution of surface waters by the ZPI formula. These values are listed in Table 2. Of interest is the much narrower range of ZPI values for each level of pollution than those proposed by Horasawa (1956). This can be explained by the fact that the microbial content of surface waters receiving effluents from aerobic treatment plants are much more consistent than that in a self-purification process after receiving raw sewage.

The other point of interest is the relative importance of the presence of nematodes in polluted waters, as indicated by the sharp increase in the protozoa:nematode ratio. In clean or remotely polluted waters without significant amounts of land runoff, there is usually absence of nematodes in gallon-size samples. As the amount of effluent received by the surface water increases, the number of nematodes increases.

Many of the large municipal water treatment plants do complete plankton analysis as a routine in water examination;

the 1971 edition of the Standard Methods recommend reporting plankton counts in numbers as well as volume-units and grouping the enumerated organisms in morphologic categories. In determining the ZPI, one needs only to filter 1-liter to 1-gallon size sample through 1 or more 8-micron membranes and wash the concentrates off the membranes with 1- to 2-ml dilution water, with the aid of a capillary pipette. The concentrated suspension is, then, examined for nematodes under X100 magnification in 1-ml amounts in a Sedgewick-Rafter counting chamber. The results are expressed as numbers of nematodes per liter. Without the need of identifying genus or species, the time required for the enumeration is much shorter than that in the routine plankton analysis.

To interpret the results for events in the course of the pollution, the ZPI value is examined in the light of the protozoa:nematode ratio and the bacteriological (virological, if any) results. For instance, a high ZPI associated with a very high protozoa:nematode ratio and high coliform count would indicate profound settling in the polluted water. A small protozoa:nematode ratio associated with a high coliform count but low ZPI would indicate that the effluent has been chlorinated prior to discharge, or the presence of toxic substance(s). The presence of nematodes in concentrations of and over 20 per liter indicates recent gross pollution irrespective of whatever the ZPI value and/or coliform count may be, unless the water is sampled at a time of large amounts of land runoff.

To illustrate the usefulness of the zoomicrobial analysis in examination of water, a study of the Ohio and Missouri rivers was conducted in the fall of 1969. Samples were taken from several sampling stations between two points of effluent discharge and examined for ZPI, nematode count, protozoa:nematode ratio, total coliform count, and fecal coliform determinations. The results obtained in this study are summarized in Table 3.

Table 3 shows that the values for ZPI and nematode count ran parallel with the coliform and fecal coliform counts in the course of pollution from the discharge point, but the nematode count dropped more rapidly than the bacterial or protozoa counts at a slow flow rate. The sharp increase in ZPI value and coliform counts in the Ohio River at a point 174 miles below the effluent discharge point resulted from another discharge of a primary effluent of Cincinnati near the Anderson Bridge. This type of treatment was indicated by the significantly lower nematode count associated with both high ZPI and coliform and fecal coli counts, since the nematode density in primary effluents was found much lower than that in effluents from secondary treatment (Chang & Kabler, 1962; Chaudhuri et al., 1965).

SUMMARY AND CONCLUSIONS

The history of the development of a pollution index on the basis of zoomicrobial contents of surface waters has been related. The various methods proposed for determining the "biological index of pollution" were based on changes in microbial composition of waters in a self-purification process after discharge of raw sewage; they are, therefore, unsuitable for

measuring the levels of pollution by effluents from waste treatment plants because the microbial population changes in receiving waters are quantitative rather than qualitative.

Since clean or remotely polluted waters predominantly contain algae with a steady increase in protozoa-nematode content as the surface waters become more polluted, a ZPI has been developed and a formula for computing its values has been presented. In applying the formula to examine samples from a large number of rivers and lakes, a scale of values has been obtained for measuring levels of pollution. A recent examination of the Ohio and Missouri River waters, by both the zoomicrobial and bacteriological methods, demonstrated that the ZPI, the nematode count, and the protozoa:nematode ratio are not only in agreement with the coliform and fecal coli results in pollution evaluation, but in addition, are indicative of some of the important events in the course of the pollution.

From these results, it is concluded that: (1) the method of zoomicrobial analysis reported in this paper is relatively simple to carry out and the values for ZPI, nematode count, and protozoa:nematode ratio are easy to obtain and are useful in determining levels of pollution, in revealing the important events in the pollution course, and in helping to decide on the extent of the treatment process; (2) the results are also helpful in judging the desirability of the waste treatment. For instance, a very high nematode content in the examined water points to a need of further process(es) beyond the secondary in the waste treatment to eliminate these worms from appearing in the finished water.

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Table 1. Population Distribution of Phytoflagellates and Zoomicrobes in Clean and in Polluted Surface Waters in the United States*

Source of Water	Number of samples examined	Population distribution of morphologic groups, mean count/l				Protozoa**
		Phytoflagellates	Nonpigmented Protozoa	Zoomicrobes	Nematodes	
Clean or remotely polluted rivers	7	45,000 $\pm$ 18,000	6,000 $\pm$ 2,000	<1	----	----
Clean or remotely polluted lakes	3	55,000 $\pm$ 21,000	4,000 $\pm$ 2,000	<1	----	----
Slightly polluted rivers	10	35,000 $\pm$ 11,000	3,000 $\pm$ 3,000	1 $\pm$ 1		8,000
Moderately polluted rivers	22	20,000 $\pm$ 7,000	12,000 $\pm$ 4,000	5 $\pm$ 2		2,400
Moderately polluted lakes	2	20,000 $\pm$ 25,000	13,000 $\pm$ 15,000	1 $\pm$ 1		14,000
Moderately heavily polluted rivers	6	10,000 $\pm$ 3,000	17,000 $\pm$ 5,000	10 $\pm$ 3		1,700
Grossly polluted rivers	5	7,000 $\pm$ 2,000	22,000 $\pm$ 8,000	30 $\pm$ 10		735

*None of these samples was taken from water polluted with stabilization pond effluent.
 **Protozoa count:nematode count ratio.

Table 2. Relationship Between the Values of ZPI and Levels of Pollution of Surface Water

Value of ZPI	Level of Pollution
1.0 - 1.2	clean or remotely polluted
1.3 - 1.5	slightly polluted
1.6 - 2.0	moderately polluted
2.1 - 3.0	moderately heavily polluted
>3.0	grossly polluted

Table 3. Results of Zoomicrobial and Bacteriological Analyses
of Polluted River Water

River examined	Sampling point (miles from discharge point)	Average flow rate (cfs)	ZPI	No. of nematodes per liter	Protozoa Nematode (ratio)	Coliform count per 100 ml	Fecal Coliform count per 100 ml
Missouri River	0 ^a	24	4.1	35	750	15x10 ⁸	29x10 ⁵
	3.5	0.5	2.2	5	2,500	9x10 ⁷	11x10 ⁶
	12.3	54,000	1.5	2	3,500	12x10 ⁴	72x10 ²
	35.5	1,200	1.4	1	3,800	5x10 ⁴	3x10 ³
	45.7	60	1.2	<1 ^b	4,500	11x10 ⁴	5x10 ³
Ohio River	0 ^c	3,500	3.6	24	950	45x10 ⁵	16x10 ⁴
	47	"	2.6	6	1,840	91x10 ³	51x10 ²
	104	"	1.5	1	2,800	61x10 ²	4x10 ²
	158	"	1.5	<1 ^d	3,500	89x10 ²	5x10 ²
	174	"	2.3	5	2,500	26x10 ⁵	48x10 ²
	222	"	1.5	<1 ^e	3,100	29x10 ²	45x10 ¹

^a Sioux City, less than 1/2 mile from point of discharge of effluent.

^b 2 nematodes per gallon

^c Huntington, W.Va., about 3/4 mile from point of discharge of effluent.

^d 2 nematodes per gallon

^e 1 nematode per gallon